

RF

<u>F72 (SEQ ID NO: 6)</u>	NFKKAAGGGGAKT	R 65-75
<u>F9 (SEQ ID NO: 7)</u>	QGSGQVNFKG	R 4-12
<u>F9 (SEQ ID NO: 8)</u>	NFKKAATPGGAAKT	R 65-75
<u>F11 (SEQ ID NO: 9)</u>	IPQQGKVTFNG	R 4-15
<u>F12 (SEQ ID NO: 10)</u>	IPEGQGKVT	R 2-12
<u>F1C (SEQ ID NO: 11)</u>	NGGTVHFKGEVVN	R5-12
<u>F1 (SEQ ID NO: 12)</u>	TTVTVNGGTVHF	R4-15

Please delete the paragraph on page 10, lines 5-18, and replace it with the following paragraph:

Results indicated the following:

<u>F serotype</u>	<u>Rilin A Sequence</u>	<u>Residue Positions</u>	<u>Homologous Protection</u>
<u>F71 (SEQ ID NO: 13)</u>	PQQGEVT	R 5-12	Yes
<u>F71 (SEQ ID NO: 14)</u>	PQQGEVA	R 5-12	Yes
<u>F71 (SEQ ID NO: 4)</u>	NRKQLQGGAAKKG	R 65-77	Yes
<u>F72 (SEQ ID NO: 5)</u>	PQQGKVT	R 5-12	Yes
<u>F72 (SEQ ID NO: 6)</u>	NFKKAAGGGGAKT	R 65-77	Yes
<u>F9 (SEQ ID NO: 15)</u>	TTVNGGTVH	R 4-12	Yes
<u>F9 (SEQ ID NO: 8)</u>	NFKKAATPGGAAKT	R 65-75	Yes
<u>F11 (SEQ ID NO: 16)</u>	IPQQGKVTFNGTV	R 4-17	Yes
<u>F12 (SEQ ID NO: 10)</u>	IPEGQGKVT	R 4-12	Yes
<u>F1C (SEQ ID NO: 11)</u>	NGGTVHFKGEVVN	R 5-15	Yes
<u>F1 (SEQ ID NO: 12)</u>	TTVTVNGGTVHF	R4-15	Yes

Please delete the paragraph on page 10, lines 20-41, and replace it with the following paragraph:

One or a combination of pilin A vaccines comprising one or more of the following amino acid sequences that correspond to published and unpublished F pilin primary sequences would be protective against ascending, non-obstructive *Escherichia coli* urinary tract infections in anatomically normal women and males:

F serotype	Pilin A Sequence	Positions	Pilin A Residue Urinary Tract Protection Potential	New or Old Claim
F71 (SEQ ID NO: 13)	PQQQGEVT	R 5-12	Pyelonephritis	New
P71 (SEQ ID NO: 14)	PQQQGEVA	R 5-12	Pyelonephritis	New
F71 (SEQ ID NO: 4)	NFKQLQQGAAKKKG	R 65-77	Pyelonephritis	New
F72 (SEQ ID NO: 5)	PQQQGKVT	R 5-12	Pyelonephritis	New
F72 (SEQ ID NO: 6)	NFKKAAGGGGAKT	R 65-77	Pyelonephritis	New
F9 (SEQ ID NO: 15)	TTVNGGTVH	R 4-12	Pyelonephritis	New
F9 (SEQ ID NO: 8)	NFKKAATPGGAAKT	R 65-75	Pyelonephritis	New
F11 (SEQ ID NO: 16)	IPQQQGKVTFNGTV	R 4-17	Pyelonephritis	New
F12 (SEQ ID NO: 10)	IPEGQQGKVT	R 4-12	Pyelonephritis	New
F13 (SEQ ID NO: 1)	PQQQGKVT	R 5-12	Pyelonephritis	Old
F13 (SEQ ID NO: 17)	AKFGGMGAKKG	R 65-65	Pyelonephritis	Old
F1C (SEQ ID NO: 11)	NGGTVHFKGEVVN	R 5-15	Cystitis	New
F1 (SEQ ID NO: 12)	TTVTVNGGTVHF	R 4-15	Cystitis	New

Please delete Table 2 on page 19 and replace it with the following Table:

TABLE 2. Primers used in this study

Primers	Oligonucleotide sequence	Description
T3	5' ATTAACCCTCACTAAAG 3' <u>(SEQ ID NO: 18)</u>	anneals to multiple cloning site of SK-
T7	5' AATACGACTCACTATAG 3' <u>(SEQ ID NO: 19)</u>	anneals to multiple cloning site of SK-
Reverse	5' AACAGCTATGACCATG 3' <u>(SEQ ID NO: 20)</u>	anneals to multiple cloning site of SK-
PGpHFD	5' ATGAGACTGCGATTCTCTGT 3' <u>(SEQ ID NO: 21)</u>	anneals to the TAC translational start region of all 4 <i>pap H</i> genes
PapHRE	5' TCCGTTCTCACAAATTCTGA 3' <u>(SEQ ID NO: 22)</u>	anneals to bp 509-528 of the <i>pap H</i> gene of pDAL201B, <i>pap-21</i> and pHUR 849, <i>pap-5</i> 210bFD
	5' CCTGAAATACGAGAAATATTA 3' <u>(SEQ ID NO: 23)</u>	anneals 93-bp upstream of the TAC translational start region of the <i>pap A</i> gene of pHUR849, <i>pap-5</i> (2)
210bRE	5' TAATATCTCGTATTTCAGG 3' <u>(SEQ ID NO: 24)</u>	the complement of 210bFD and anneals to the same 93-bp region as described for 210bF
FOR210b	5' TGGACTGGTATAACAATCGA 3' <u>(SEQ ID NO: 25)</u>	anneals 2.9 kb upstream of the TAC translational start region of the <i>pap H</i> gene of pDAL210B, <i>pap-21</i>
200aRE	5' TCCGTTTCGCACAATTCTGA 3' <u>(SEQ ID NO: 26)</u>	anneals to bp 511-528 of the <i>pap H</i> gene of pDAL210B, <i>pap-17</i> , and <i>pap 200a</i> , respectively
PapFOR ^a	5' AGT <u>GGATT</u> CATGCAGCATTCT AGAAA 3' <u>(SEQ ID NO: 27)</u>	anneals to bp 258-270 of the <i>pap A</i> gene of pHUR849, <i>pap-5</i> (2)
FORSEQ	5' TGGACCTCCTGAGCTA 3' <u>(SEQ ID NO: 28)</u>	anneals to bp 456-474 of the <i>pap A</i> gene of pHUR849, <i>pap-5</i> (2)
PapREV ^b	5' GGGGCAGCCCTGCCGTCCCAA AT 3' <u>(SEQ ID NO: 29)</u>	anneals to bp 122-142 of the <i>pap H</i> gene of pHUR849, <i>pap-5</i>
REVSEQ	5' AAACACCATGAAACACACA 3' <u>(SEQ ID NO: 30)</u>	anneals to bp 41-61 of the <i>pap H</i> gene of pHUR849

^a contains a single Bam HI restriction site single underlined.

^b contains a single Sma I blunt end restriction site double underlined.

Please delete the paragraph on page 22, line 5 to page 23, line 6 and replace it with the following paragraph:

Nucleotide Sequences and Deduced *PapH* Primary Structures

The plasmids pHUR849 (*pap-5*), pDAL201B (*pap-21*), pDAL210B (*pap-17*) and, pDAL200A (*pap-200A*), in *E. coli* strain HB101 express digalactose-binding of the serotypes F13, F7₁, F7₂ and F9, respectively. The *pap* gene cluster responsible for regulation and biogenesis of these pili from *E. coli* strains J96, C1212 and, 3669 is 1U. diagrammed in FIG. 1. Sequence analysis of *papH* genes from pDAL201B (*pap-21*), pDAL210B (*pap-17*) and, pDAL200A (*pap-200A*), was compared to the known nucleotide sequence of *papH* gene of

pHUR849 (*pap-5*) (3). FIG. 2 shows a single 588-bp open reading frame with the same polarity as *papA* (2, 4). Analyses of these *papH* sequences revealed many typical features of prokaryotic gene organization. All four *papH* gene sequences contained a potential ribosome-binding sites, ATG initiation codon signal sequence, and a TGA termination codon. A potential initiation codon ATG at position -22, preceded by a sequence corresponding to -AGGGT, which showed homology to ribosome-binding sites, was found 13-bp upstream in all four *papH* sequences. A protein initiated here and ending at the TGA triplet at position 586 would encode a 195 amino acid polypeptide with a calculated molecular weight of 21.9 kd. The mature *PapH* protein contains 173 amino acid residues. The NH₂-terminal amino acid sequence of the open reading frame has all the features of a signal peptide sequence. The deduced putative signal sequence for the *papH* was located 22 codons upstream of their terminal Ala (FIG. 2). These sequences contained a highly hydrophobic region comprising an amino acids stretch of Ser-Val-Pro-Leu-Phe-Phe-Phe (residues -17 to -11 of SEQ ID NO: 32). There was a positively charge amino acid residue (Arg) at the position -21. The suggested cleavage sites between Ala -1 and gly +1 conforms to rules of prokaryotic signal cleavage sites and was similar to most other bacterial genes (12). In addition, the final *papH* deletion derivatives, pKD849-5 (*pap-5*), pKD201B (*pap-21*), pKD210B-1 (*pap-17*) and pKD200A-8 (*pap-200A*), were also sequenced. In addition, sequencing into the *papA* and *papC* genes which flank the *papH* gene (FIG. 1) of all four *papH* deletion derivatives was carried out in order to insure that all three genes were in frame. Finally, the codon usage of the *papH* genes of pDAL201B, pDAL210B and, pDAL200A, and *papH* gene of pHUR849 were analyzed using a codon frequency computer program (13). The pattern of codon utilization was not significantly different among the genes.

In the Figures:

NE
Please replace Figure 5 with replacement sheet Figure 5 submitted herewith to correct a typographical error in Figure 5B at position 58. Position 58 should recite a "Q" instead of an "O."

In the Claims:

BC
1. (Presently Amended) An immunogenic composition comprising dissociated pili from a α -D-Galp-(1-4)- β -D-Galp (Gal-Gal) binding pilus-producing *Escherichia coli* bacteria, said pili comprising at least one immunogenic peptide inserted into the immunodominant region